

Analysis of Miro GTPase Mediated Mitochondrial Movement

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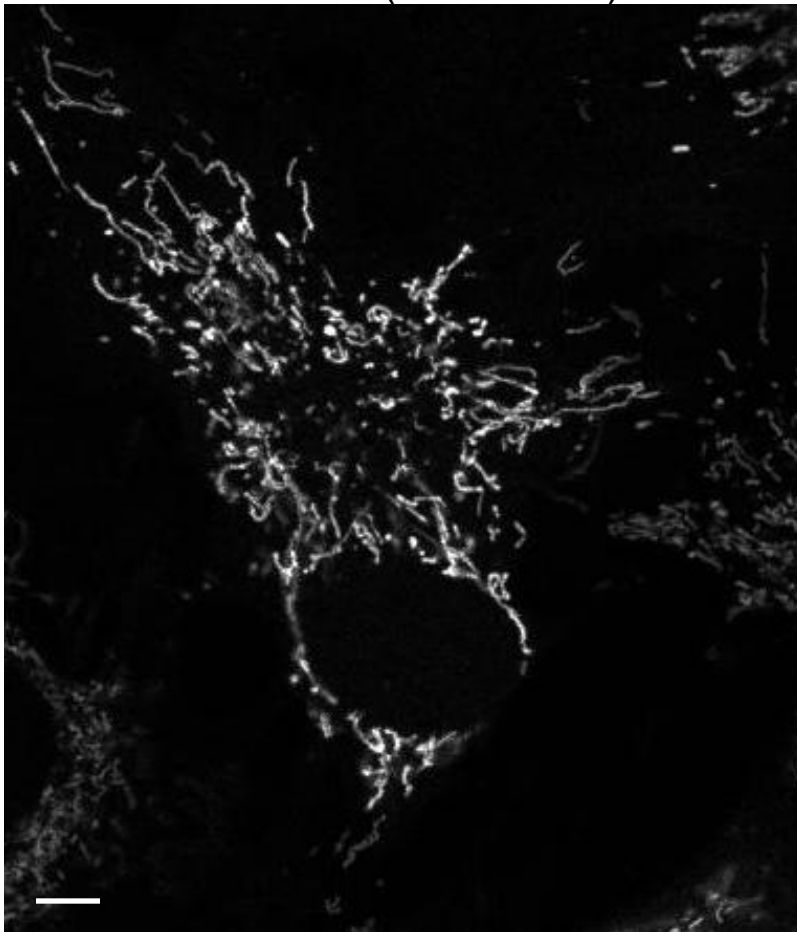
Janet Shaw Lab

Biochemistry Department

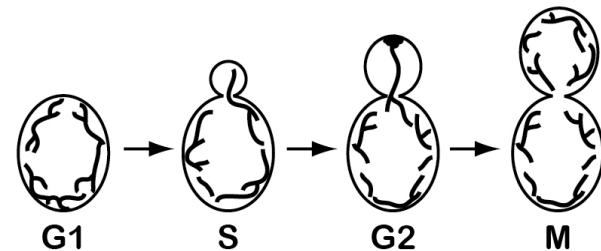
May 11, 2012

Mitochondrial Movement is a Conserved Biological Function

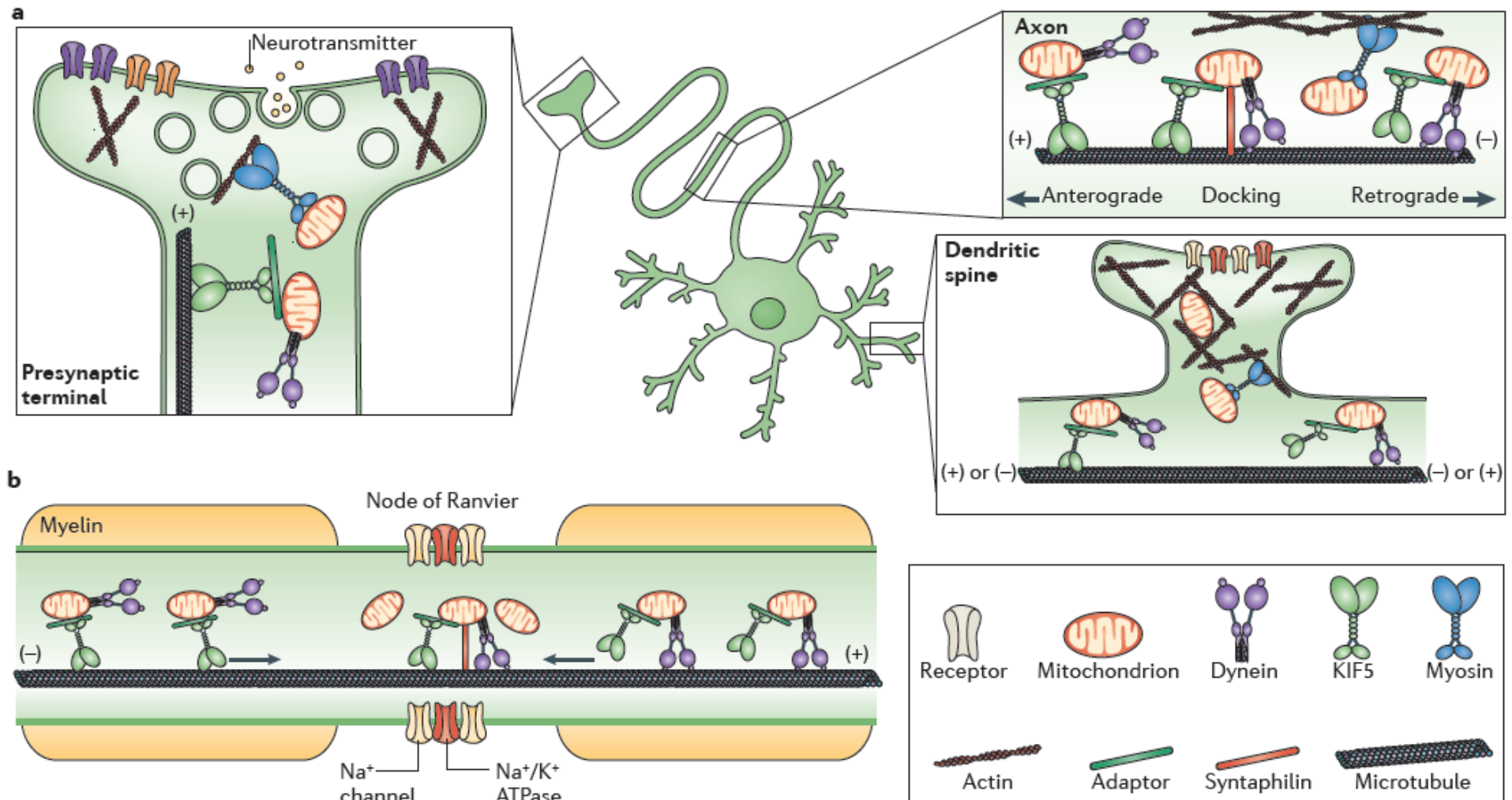
Mitochondria in mouse fibroblast
2.8 minutes (7sec/frame)

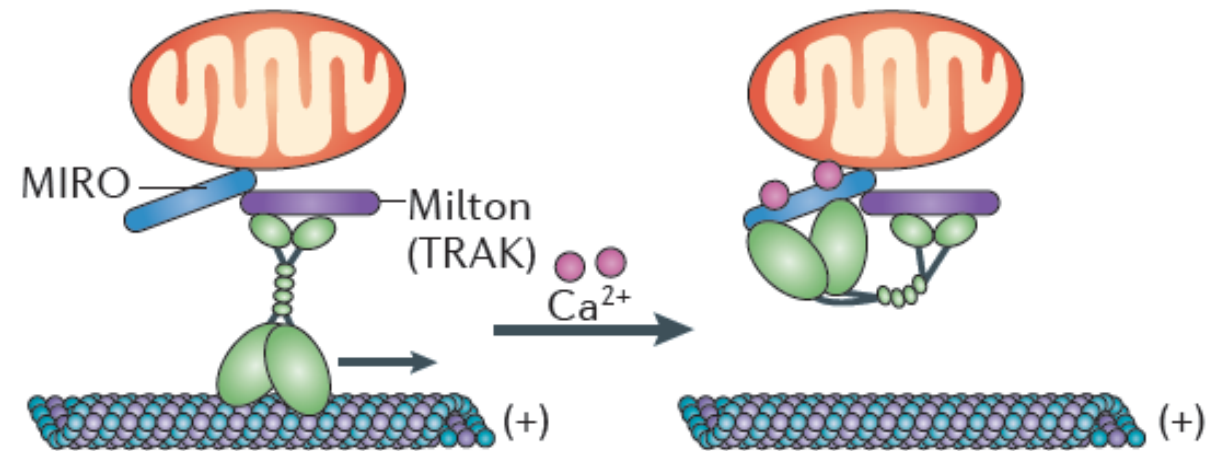
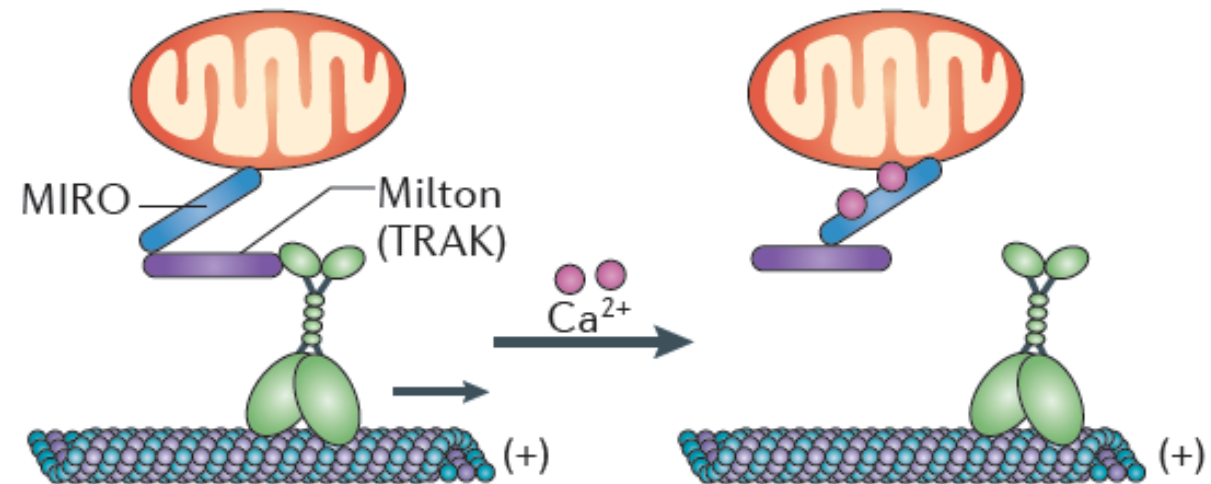


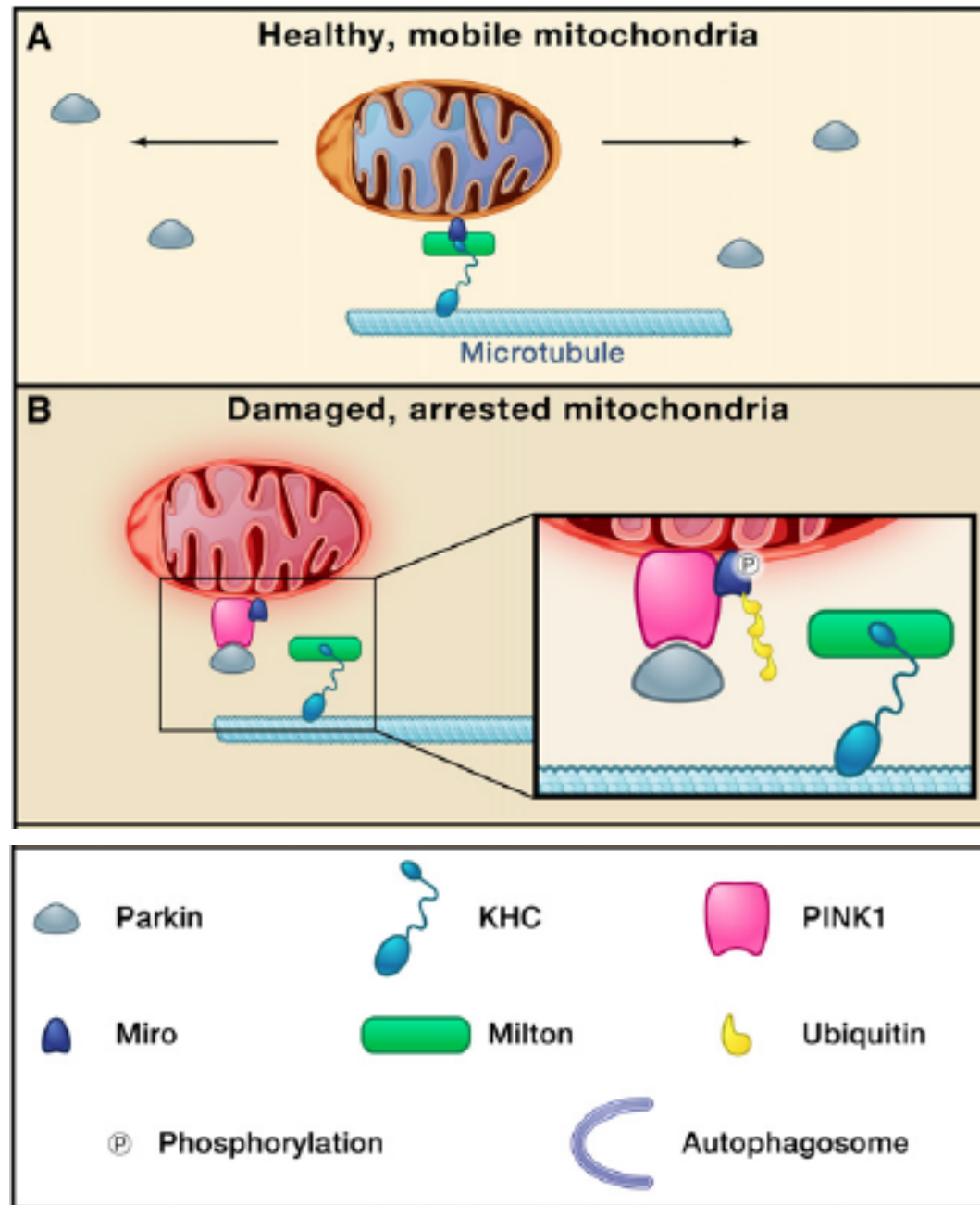
Mitochondria in budding yeast
Jacob lab, 214 minutes



Mitochondrial Movement in Neuron



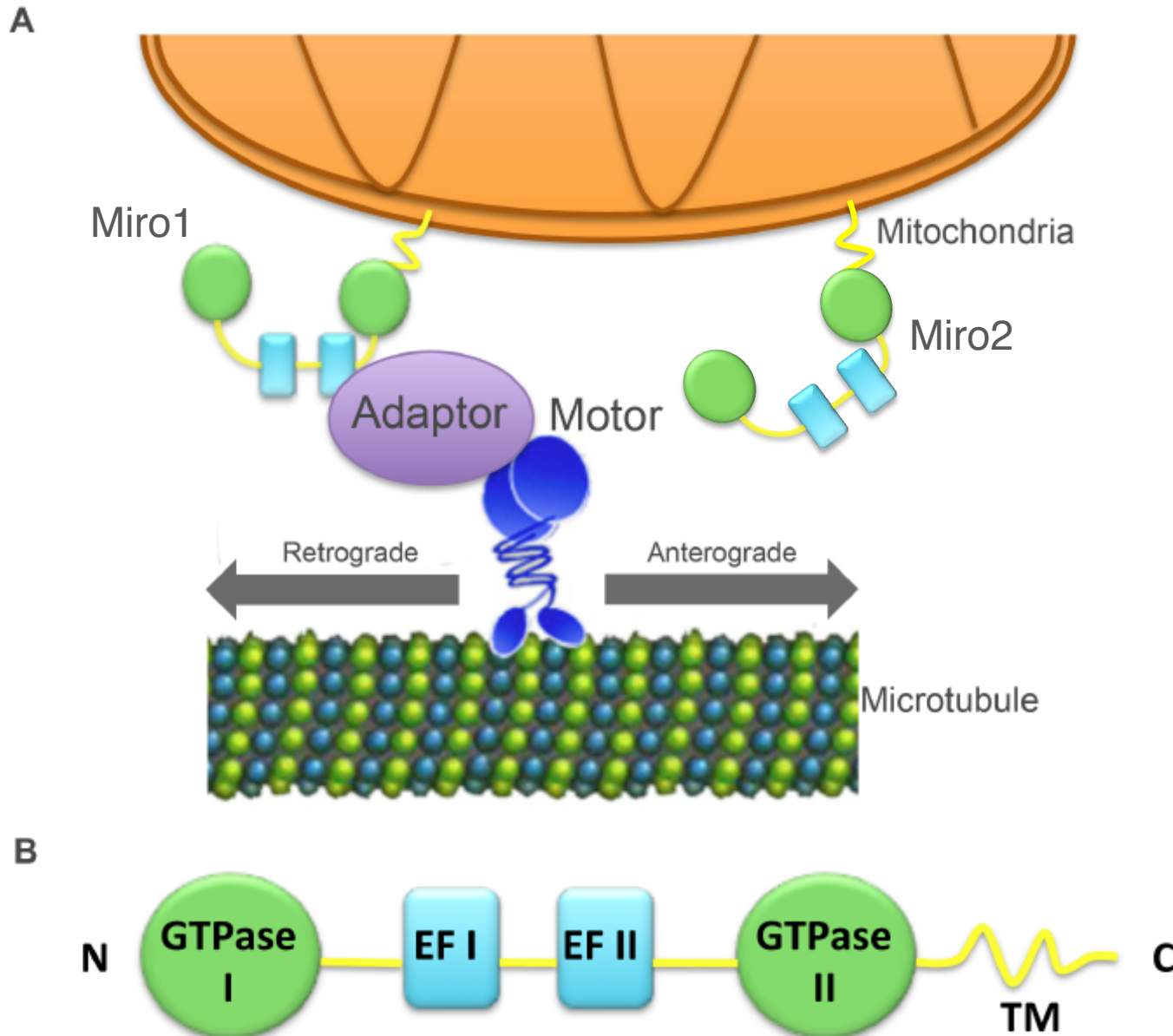
c**d**



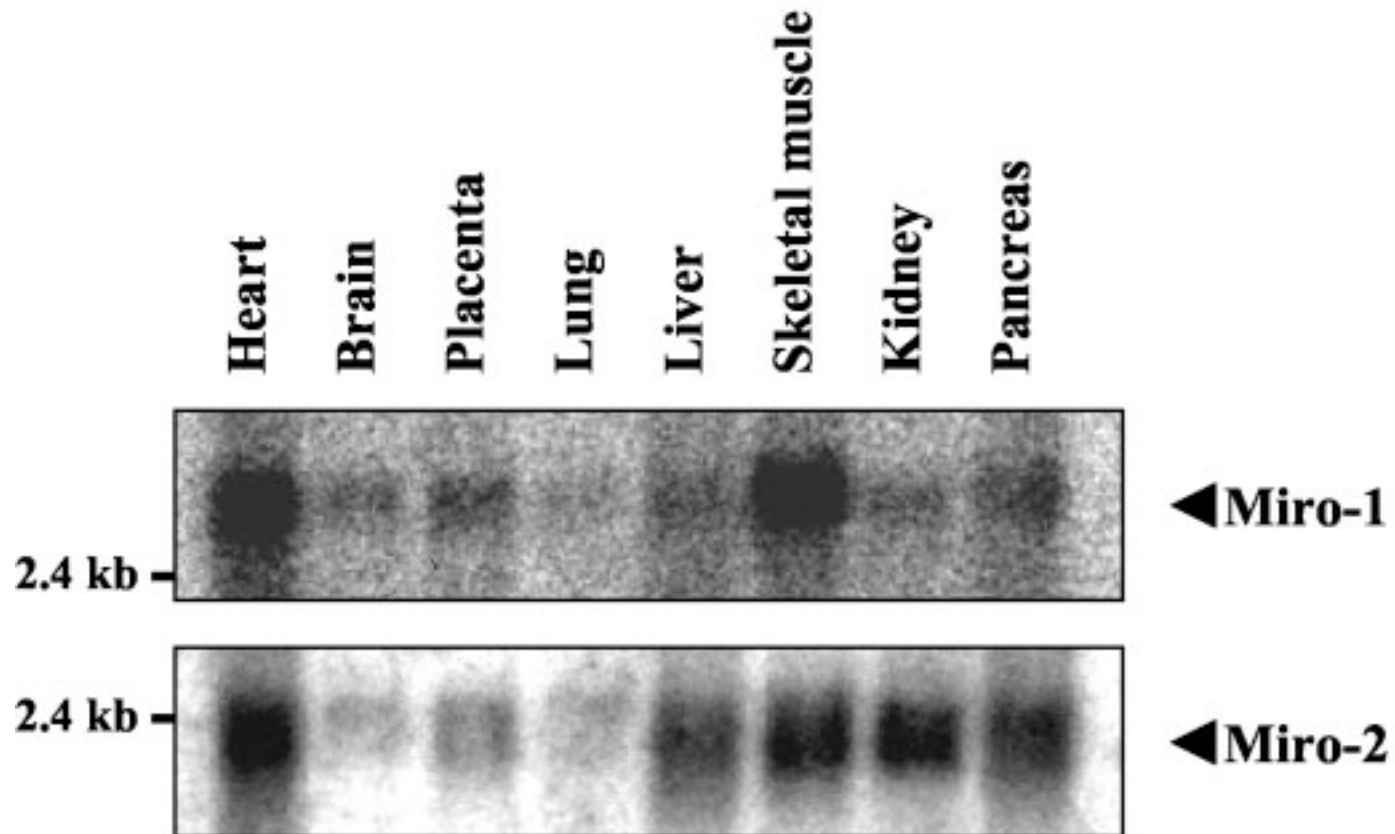
Neurodegenerative Diseases Linked to Mitochondrial Movement Defects

- Parkinson's disease
 - Pink1/Parkin target Miro to arrest damaged mito
- Alzheimer's disease
 - Exposing neurons to amyloid- β results in impaired mitochondrial movement
- Amyotrophic Lateral Sclerosis
 - Loss of or $\uparrow$ damaged mitochondria in distal motor axons
- Huntington's disease
 - mutant HTT disrupts the formation of transport complexes and impairs mitochondrial movement

Miro is a Conserved Mito OM Receptor



Miro1 and Miro2 Transcripts are Ubiquitously Expressed

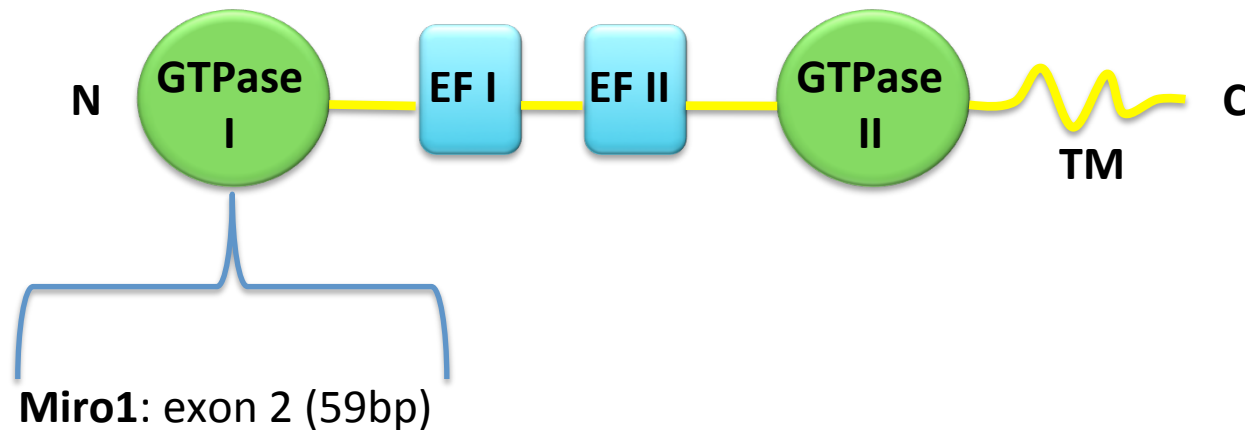




Is Miro-mediated mitochondrial movement required for neuro-development?

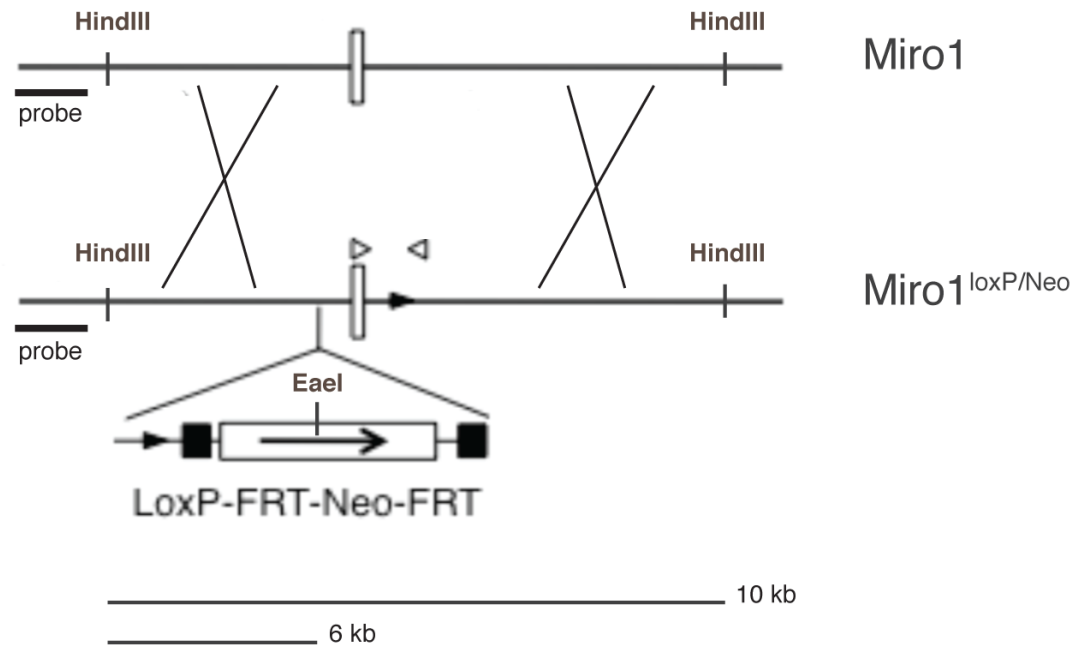
Is Miro-mediated mitochondrial movement required for neuron maintenance?

Generating Miro1 KO Mice

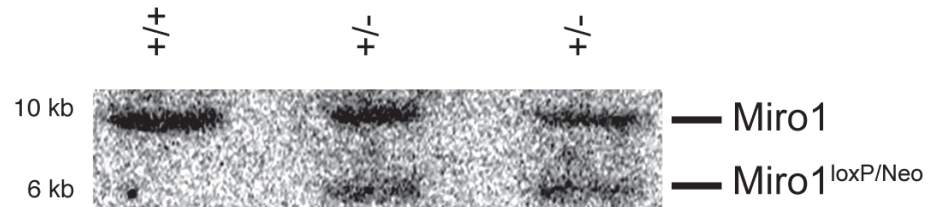


Generating Miro1 KO Mice

A



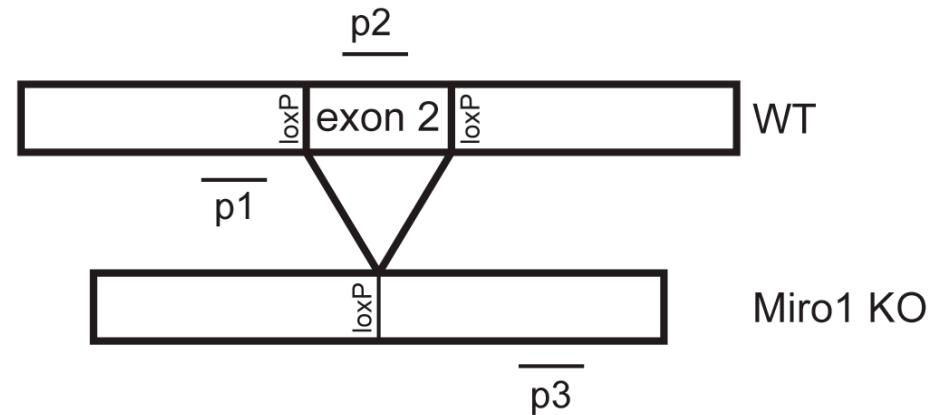
B



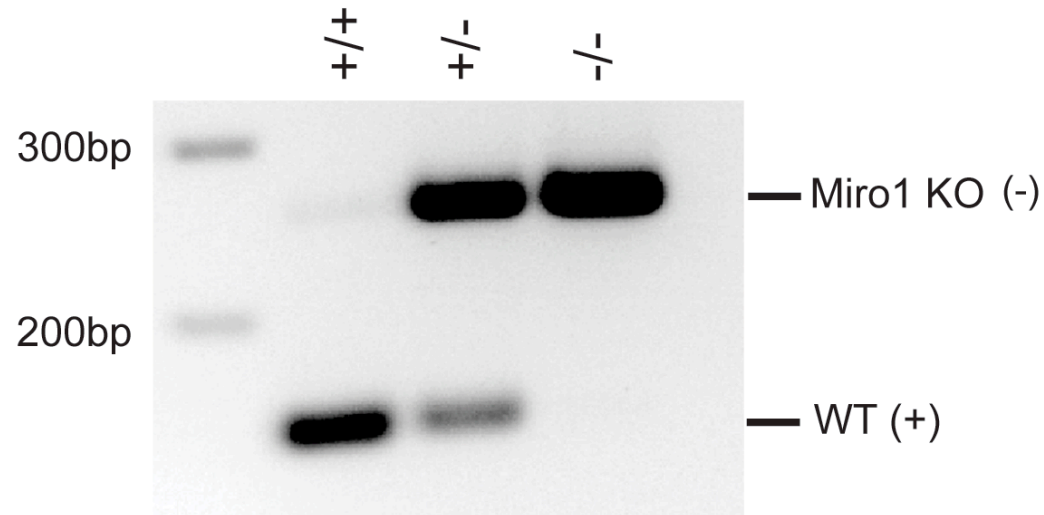
Miro1 KO Mouse

Miro1^{+/-} x Miro1^{+/-}

A



B



Miro1 KOs are Postnatally Lethal

Miro1^{+/-} x Miro1^{+/-}

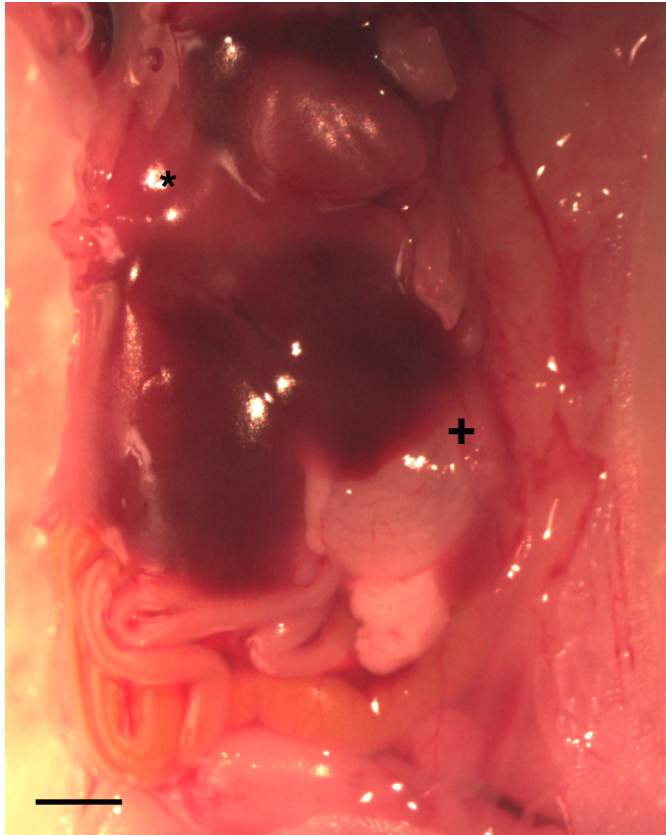
Gestational Age	Total # of Embryos	# Embryos			% (#) Dead Pups		
		+/+	+/-	-/-	+/+	+/-	-/-
E14.5	8	1	5	2	--	--	--
E18.5	19	2	10	7	--	--	--
P0.5	33	5	21	6	20 (1)	10 (2)	100 (6)

Miro1 ^{-/-}

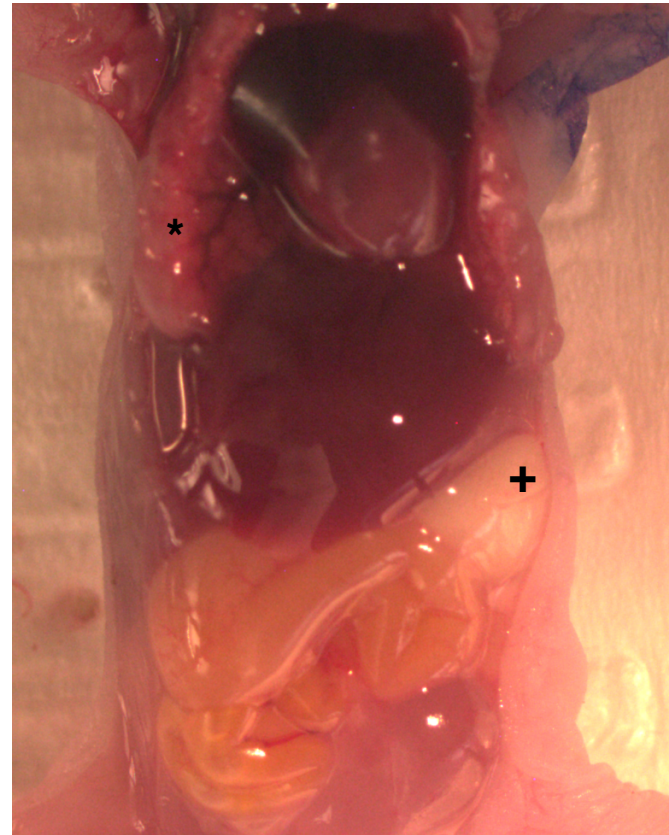
Miro1 ^{+/-}



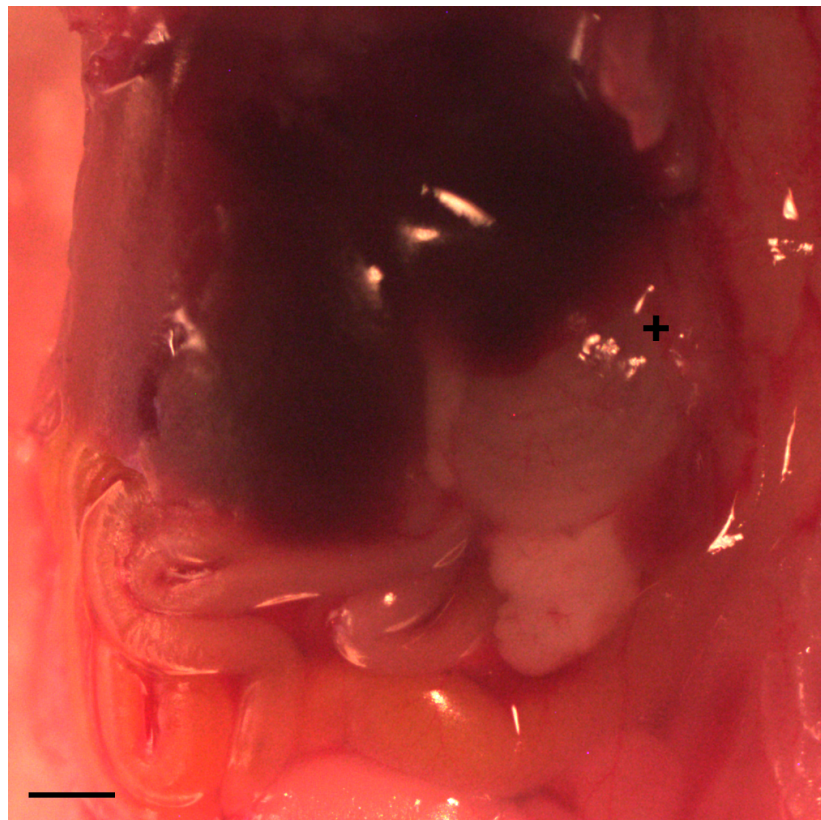
Miro1 ^{-/-}



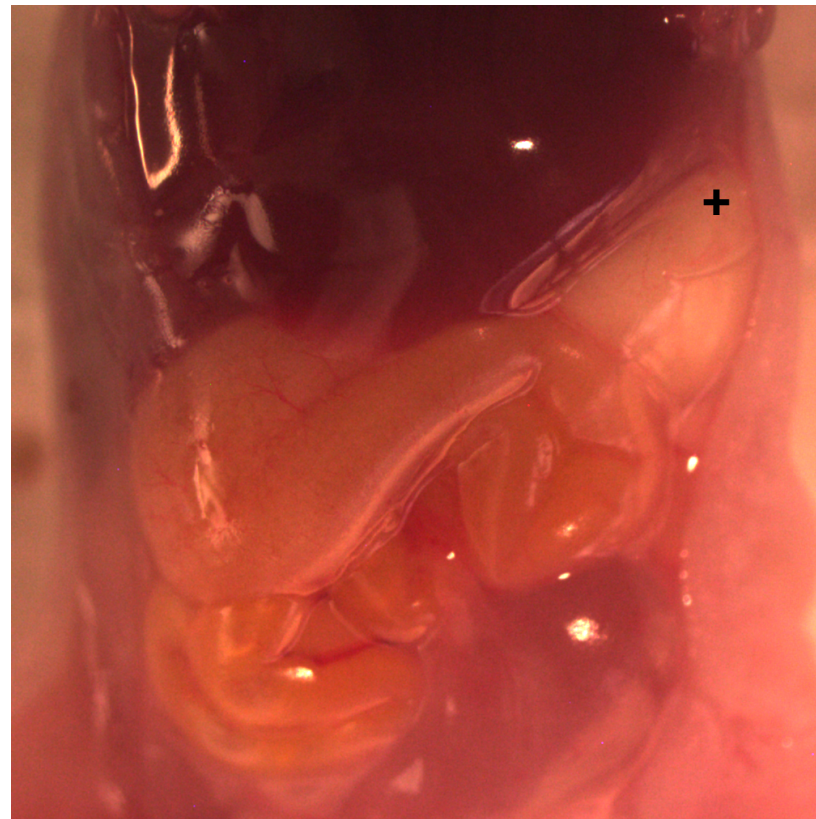
Miro1 ^{+/+}



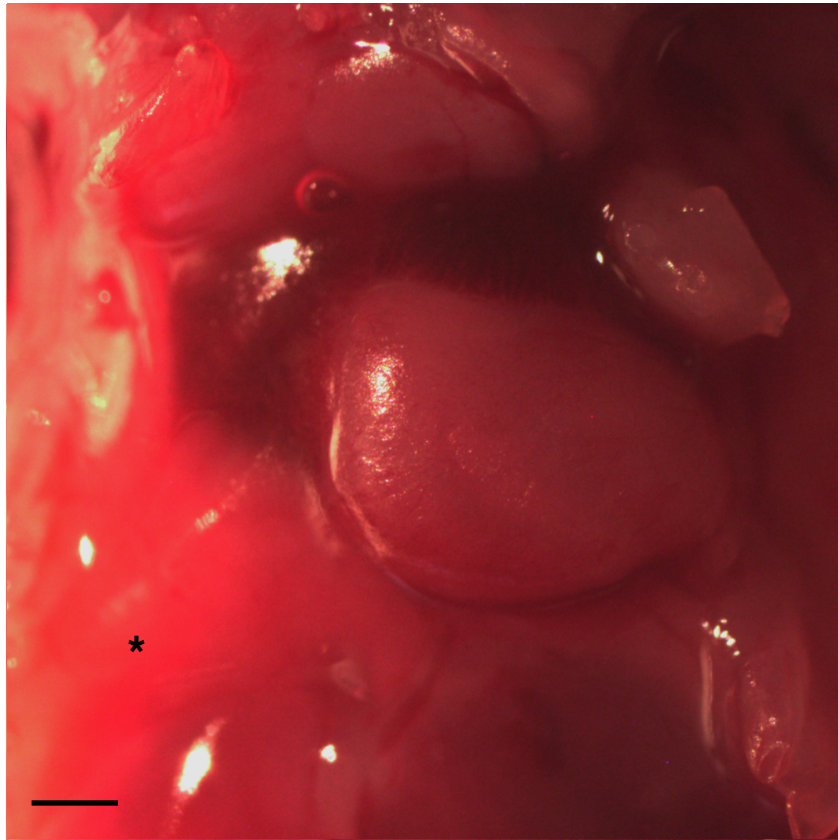
Miro1 ^{-/-}



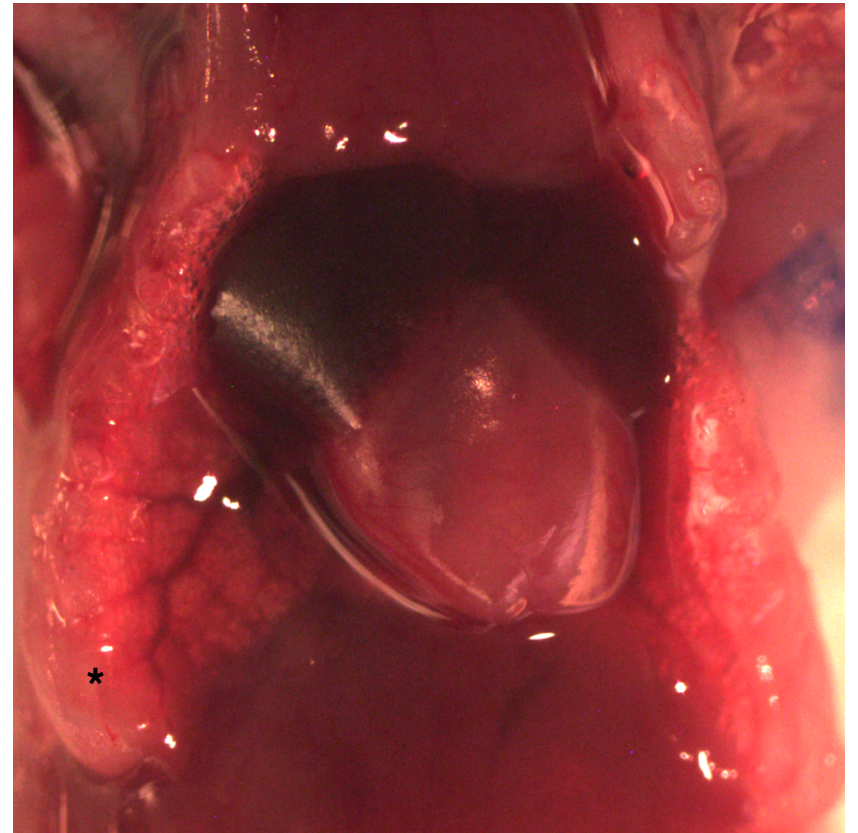
Miro1 ^{+/+}



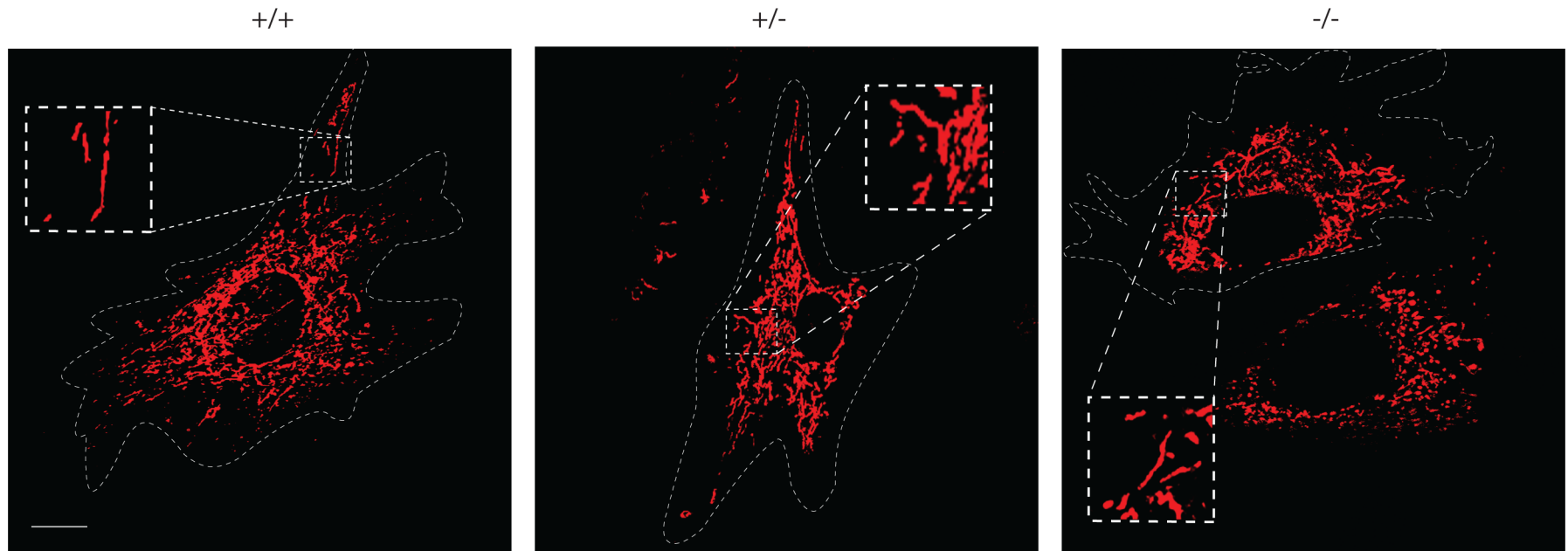
Miro1 ^{-/-}



Miro1 ^{+/+}

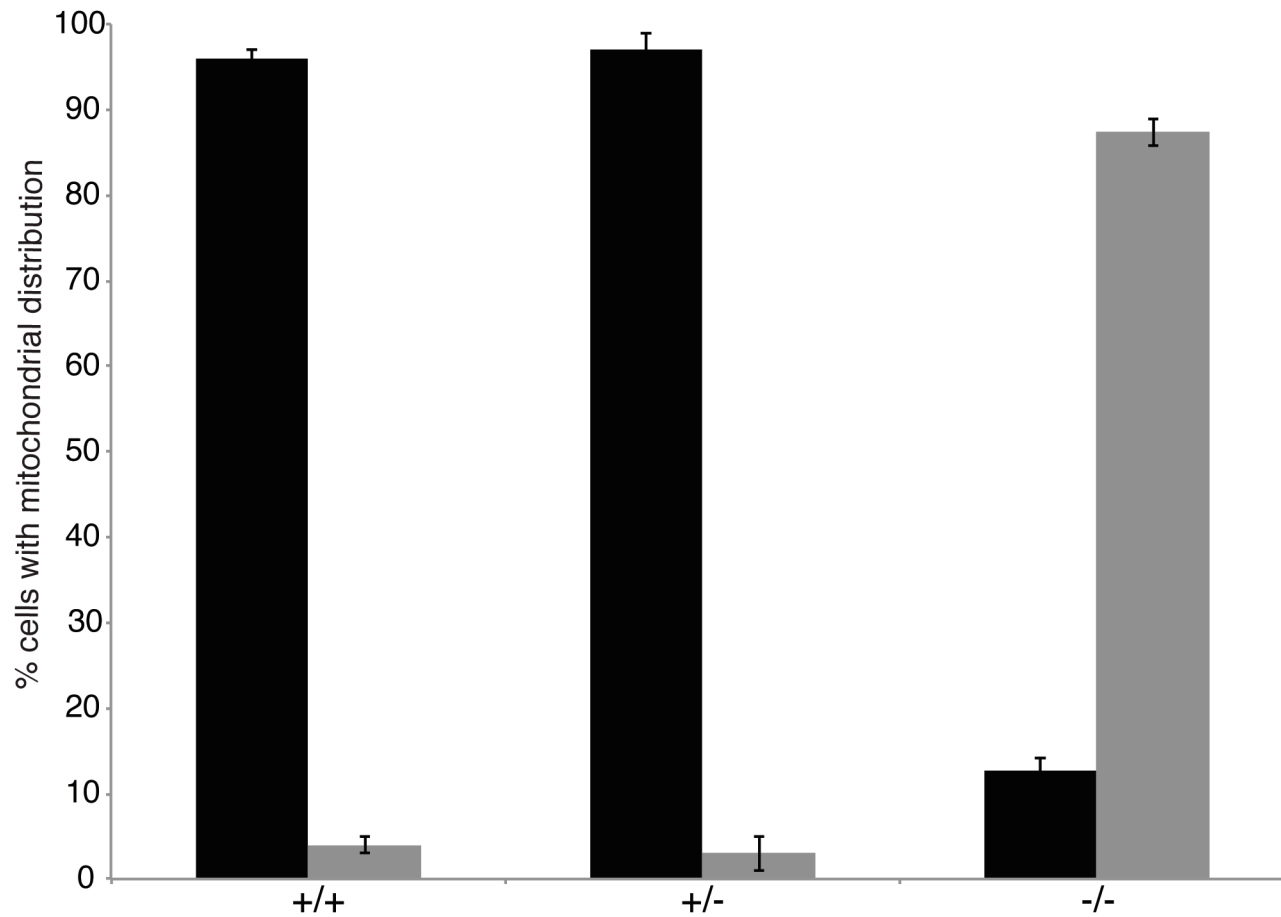
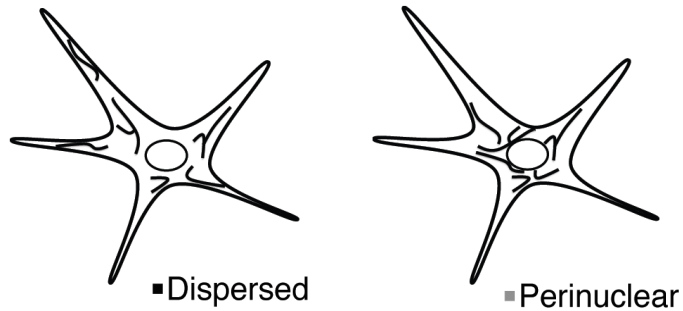


Mitochondria in Miro1^{-/-} are Perinuclear

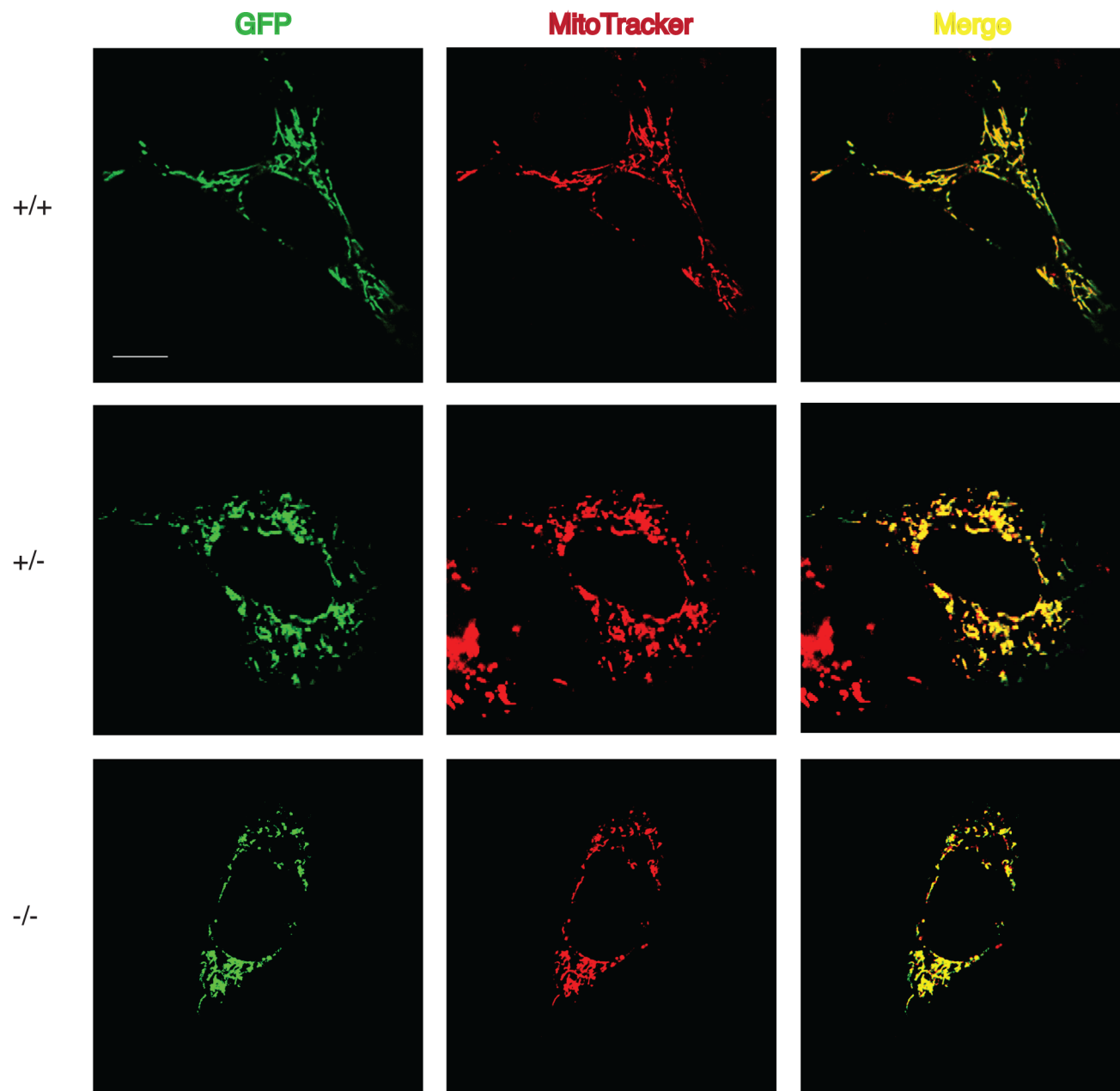


*~5 hours Pyruvate DMEM

Miro1^{-/-} have Mito Distribution Defect



n=300



Current experiments....

- Make Miro1 & Miro2 antibodies
- Miro2 qPCR
- Mito movement recordings in cortical neurons
- Test for axon and dendritic outgrowth by immunofluorescence
- Express Miro mutants and WT to test for mito distribution rescue
- Knockdown Miro2 in Miro1^{-/-} MEFs
- Examine mito/ER contacts by fluorescent microscopy
- mtDNA copy number qPCR

Set up conditional Miro1^{-/-} KOs

- Eno2-cre (neuron specific)
- Mnx-cre (motor neuron specific)
- MHC-cre (cardiac specific) ✓

Miro1^{F/+} MHC^{cre/+} x Miro1^{+/-} cross

Gestational Age	Total # of Embryos	# Embryos			% (#) Dead Pups		
		+/+	+/-	-/-	+/+	+/-	-/-
P0.5	17	7	6	4	0 (0)	25 (2)	25 (1)

+/+ = WT/WT or WT/F with no cre

+/- = WT/- or F/- with no cre

Future Experiments

Experiments to do using Miro1 ^{-/-} MEFs

- Seahorse to measure metabolic function
- Test Miro1 and Miro2 TAP tag constructs

Experiments to do using Miro1 ^{-/-} animals

- Histology
- EM

Experiments to do using Miro1 ^{-/-} neurons

- Synaptic release study
- Electrophysiology recordings
- Test for Mito association to motor proteins

The Shaw Lab:

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The Tantin Lab:

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Thank you!

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